cytosolic side of the membrane. Disruption of this electrochemical equilibrium causes V_m to become more positive or more negative relative to its resting state, referred to as ''depolarization'' or ''hyperpolarization,'' respectively. Changes in membrane potential have proven to be pivotal not only in normal cell cycle progression, but also in malignant transformation. Using polystyrene nanoparticles as a model system, we use a combination of fluorescence microscopy and flow cytometry to measure changes in membrane potential in response to nanoparticle binding to the plasma membrane. We find that cationic nanoparticles depolarize both CHO-K1 and HeLa cells. The cellular binding of anionic nanoparticles does not lead to a discernible trend in altered membrane potential. Maintenance of the resting membrane potential depends on the presence of two-pore-domain potassium ''leak'' channels, which allow for outward diffusion of potassium ions along their concentration gradient. Using an assay that tests the diffusion of ions through these potassium channels, we observe reduced permeability of the channels when cells are treated with nanoparticles. Based on a dynamical system model of the cell, we conclude that this loss of permeability likely results from physical blockage of the channel itself by the particle. Prevention of potassium ion efflux due to blocked channels causes accumulation of positive charge inside the cell, resulting in a depolarized membrane. By understanding the ways in which nanoparticles can be utilized to selectively generate cellular responses, we can begin to consider them as active species that may alter the very systems they are currently designed to probe.

935-Plat

Electro-Wetting of a Hydrophobic Gate in a Biomimetic Nanopore

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Nanopores in membranes have a range of potential applications. Biomimetic design of nanopores aims to mimic key functions of biological pores within a stable template structure. Molecular dynamics simulations have been used test whether a simple β-barrel protein nanopore can be modified to incorporate a hydrophobic barrier to permeation. Simulations have been used to evaluate functional properties of such nanopores, using water flux as a proxy for ionic conductance. Potential of mean force calculations have been used to calculate free energy landscapes for water and for ion permeation in pore models. These studies demonstrate that a hydrophobic barrier can indeed be designed into a β-barrel protein nanopore, and that the height of the barrier can be adjusted by modifying the number of consecutive rings of hydrophobic sidechains. A hydrophobic barrier prevents both water and ion permeation even though the pore is sterically unoccluded [1].

A clear prediction of the hydrophobic gating model is that of electro-wetting of the gate should occur, i.e. the pore can be functionally opened by applying a high transmembrane voltage. This has been seen experimentally in studies of hydrophobically-gated solid state nanopores [2]. We have explored electro-wetting of our model of a hydrophobic gate in a simple β-barrel protein nanopore using atomistic molecular dynamics simulations with either a constant field applied or with the recently developed 'computational electrophysiology' approach [3] to model a voltage difference across the pore and bilayer. The results of both methods demonstrate voltage-dependent de-wetting in these pores.

1. J. Trick et al (2014). Designing Hydrophobic Barriers into Biomimetic Nanopores (submitted)
2. M. R. Powell, et al. (2011). Nature Nanotechnology, 6, 798-802.
3. C. Kutzner et al. (2011). Biophys J, 101, 809-817.

936-Plat

Deformation of MCF-7 Cells in Micropores with Undulating Diameter

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Pores used in current resistive-pulse experiments were shown to measure size and surface charge of translocating objects. With the addition of the undulation of the pore opening diameter it is possible to simultaneously characterize the size and mechanical properties of the object that has been passed through the pore. Here we will discuss in detail the characterization of a cell's size and mechanical properties by examining the resistive-pulse of each individual cell. The cells translocated the pore electrokinetically and no external pressure difference was applied. Previous experiments with polystyrene and hydrogels particles as well as numerical modeling of electroosmotic fluid flow in our pores revealed existence of pressure drops along the pore axis. The local pressure gradients were predicted to deform biological cells even if the pore opening was larger than the cell at any axial position. Polystyrene particles suspended in a solution of HBSS and Tween 80 were first passed through single undulating micropores to measure their topography. MCF-7 cells were suspended in a solution of HBSS and pluronic, and passed through the same pores. Deformation of the cells was observed as a change of the relative amplitude of the pulse sub-peaks compared to the signature obtained with the hard polystyrene particles. Viability of the cells after their passage through the micropore was also established.

937-Plat

Fingerprinting Single Living Cells with Molecular Precision

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The secretome of a single living cell contains the totality of its secreted proteins,(1) and therefore can act as a fingerprint by which to identify cell type. Although between 10 % and 20 % of the human genome encodes proteins that are secreted, measuring the secretome from an individual living cell is challenging as the secreted proteins are present in vanishingly small concentrations due to the very large dilutions involved.

However, a nanopore is able to detect single proteins through the distinctive blockage profile that develops in the ionic conductance current when a protein passes through the nanopore.(2,3) Using a synthetic nanopore in a silicon membrane we investigate the distinctive blockage patterns, in essence the fingerprint, that arise from a single living cell. The cell is placed in proximity to the nanopore using optical tweezers and held stationary. The ionic conductance current is measured across the nanopore, and translocation events (distinct blockage currents) are observed and measured. When the events are plotted in scatter plots (as dwell time versus average blockage current) the distinct fingerprint of individual cells can be observed. For instance, lymphoma cells (U937) and breast cancer cells (MCF7) produce distinct event patterns that enable them to be distinguished. This shows for the first time cell identification based entirely on the secretome, measured using a simple, non-invasive, non-destructive nanopore.

(1) Skalnikova, H.; Motlik, J.; Gadher, S.; Kovarova, H. Proteomics, 2011, 11 691-708.
(2) Nelson, E. M.; Kurz, V.; Shim, J.; Timp, W.; Timp, G. Analyst, 2012, 137, 3020.
(3) Kurz, V.; Nelson, E. M.; Shim, J.; Timp G. ACS Nano, 2013, 7(5), 4057-4069

938-Plat

Mechanical Modulation of Enzyme Activity by Rationally Designed DNA Tweezers: From the Ensemble to the Single-Molecule Level

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Switchable nanomachines provide a platform to control dynamic functional states by altering distances at the nanoscale on demand. Recently, a tweezers-like DNA device was used to control the activity of an enzyme/cofactor pair juxtaposed on the two arms of the tweezers. Initial studies focused on bulk properties of the tweezers-mediated reactions, and hence lacked insight into the mechanism of enzymatic activation. Here, we used site-specific fluorophore labeling of the tweezers to monitor the arm-to-arm distance through single-molecule fluorescence resonance energy transfer (smFRET). Consistent with AFM measurements, smFRET showed that the tweezers only partially close in the proposed ''closed'' state and exhibit conformational sub-states. MD simulations showed bending and twisting of the tweezers arms, rationalizing the sub-states. Additionally, smFRET experiments on the isolated Holliday junction hinge suggested that the isomer resulting in the tightest closing of the tweezers (isomer-I) is relatively disfavored, further explaining the only partial closing. We rationally improved the closing by increasing the stem length of the DNA hairpin bridging and actuating the tweezers from 3 to 4 base pairs, and by redesigning Holliday junction(s) of the tweezers to favor the optimal isomer-I. The performance of the new tweezers was quantitatively assessed by juxtaposing glucose-6-phosphate dehydrogenase (G6pDH) with its cofactor NAD^+ on the tweezers arms and measuring the G6pDH activity through a coupled enzymatic cascade. Using our optimized tweezers, we were able to enhance the bulk activity of G6pDH upon tweezers closure to up to ~12-fold. Currently, we are exploring the tweezers-manipulated enzymatic reaction